ELSEVIER

European Journal of Pharmacology 315 (1996) 187–194

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Cardiovascular effects produced by *R*-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin in the preoptic area of conscious rats

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Received 11 April 1996; revised 29 July 1996; accepted 2 August 1996

Abstract

Experiments were designed to test the hypothesis that activation of forebrain 5-HT_{1A} receptors elicits cardiovascular responses. The microinjection of *R*-(+)-8-hydroxy-2-(di-*n*-propylamino) tetralin [(+)-8-OH-DPAT], a selective 5-HT_{1A} receptor agonist, in the preoptic area of conscious rats increased blood pressure and heart rate at doses of 0.2–20 nmol; lower doses (0.002 and 0.02 nmol) were ineffective. The concomitant administration of methiothepin, a non-selective 5-HT receptor antagonist, into the preoptic area attenuated the responses. In addition, the tachycardia elicited by (+)-8-OH-DPAT was abolished by the peripheral β-adrenoceptor antagonist sotalol, but not by atropine methyl nitrate. Finally, the tachycardia, but not the hypertension, was also produced by (+)-8-OH-DPAT in urethane-anesthetized rats. These results suggest that activation of 5-HT_{1A} receptors in the preoptic area or an adjacent region of the forebrain produces: (1) an increase in heart rate consistent with sympathoadrenal activation; and (2) an increase in blood pressure which might be the result of sympathoexcitation or secondary to behavioral arousal.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT (*R*-(+)-8-hydroxy-2-(di-*n*-propylamino) tetralin); Blood pressure; Heart rate; Preoptic area; Forebrain

1. Introduction

It is generally accepted that the central 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicits hypotension and bradycardia by actions primarily in midbrain raphé and brainstem nuclei (McCall and Clement, 1994). Recently, it was demonstrated that low doses of serotonin and selective 5-HT_{1A} receptor agonists administered into the lateral cerebral ventricle in rats produce the opposite cardiovascular responses: hypertension and tachycardia (Anderson et al., 1992; Dedeoğlu and Fisher, 1991, 1994). It was suggested that these responses were mediated by activation of 5-HT_{1A} receptors in the forebrain. Earlier studies demonstrated that serotonin can act within the preoptic area and/or the anterior hypothalamus to affect the cardiovascular system (Kuhn et al., 1980; Smits et al., 1978; Smits and Struyker-Boudier, 1976; Wolf et al., 1981). For example, electrical stimulation of the dorsal raphé nucleus increased blood pressure and heart rate; the effects were abolished by the application of non-selective 5-HT receptor antagonists into the preoptic area/anterior hypothalamus. Although these studies do not necessarily implicate the 5-HT_{1A} receptor, they provide evidence that there are forebrain sites in which activation of 5-HT receptors can elicit hypertension and tachycardia.

Experiments were designed to investigate the effects of the full, selective 5-HT_{1A} receptor agonist (+)-8-OH-DPAT on blood pressure and heart rate when microinjected directly into the preoptic area. This route of administration would limit the site of action to the forebrain, thereby reducing or eliminating the likelihood of activating 5-HT_{1A} receptors in the brainstem as would occur following i.c.v. injections. It was found that the microinjection of (+)-8-OH-DPAT into the preoptic area in conscious rats

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PII S0014-2999(96)00626-7

elicited small and variable increases in blood pressure and modest consistent increases in heart rate. Additionally, the tachycardia was reduced by concomitant microinjection of the non-selective 5-HT receptor antagonist methiothepin and blocked by pre-treatment with the peripheral β-adrenoceptor receptor antagonist sotalol.

2. Materials and methods

2.1. General methods

Experiments were performed in male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA). The animals were housed in temperature-controlled quarters (22.5°C) on a 12-h light/dark cycle with ad libitum access to rat chow and tap water. 9 days before experiments in conscious animals, rats (240–270 g) were anesthetized with a mixture of acepromazine and ketamine (1.3 and 105 mg/kg i.p., respectively). Stainless-steel guide cannulas (26 gauge; Plastic Products, Roanoke, VA, USA) were implanted stereotaxically directed 1 mm above the preoptic area using the following coordinates: +8.0 mm anterior from interaural zero, −0.6 mm lateral from midline and +2.1 mm ventral from interaural zero. Approximately 1 week later, a polyethylene catheter filled with heparinized saline (50 U/ml 0.9% NaCl) was inserted into the abdominal aorta through the left femoral artery and routed s.c. to exit at the nape of the neck (Alper and Schmitz, 1996; Pérgola et al., 1993; Pérgola and Alper, 1991, 1992). The rats were allowed to recover for at least 2 days following the catheter surgery. In 1 set of rats (Expt. 5, Section 3.6), catheters were also inserted through the femoral vein into the vena cava and exteriorized along with the arterial catheter. Except for the initial desensitization study (Expt. 1, Section 3.2), rats were used only 1 time. In the only experiment performed under anesthesia (Expt. 3, Section 3.4), stereotaxic surgery and arterial and venous catheter implantations were performed sequentially under acepromazine-ketamine anesthesia 48 h prior to the experiment. On the day of the experiment, the rats were anesthetized with urethane (1.2 g/kg i.v.) and approximately 2 h later were administered either artificial cerebrospinal fluid (aCSF; vehicle) or (+)-8-OH-DPAT into the preoptic area.

2.2. Experimental protocols

The exteriorized arterial catheter was connected to a P10EZ pressure transducer (Viggo-Spectramed, Oxnard, CA, USA) for continuous measurement of pulsatile blood pressure. Conscious rats were then allowed at least 60 min to acclimate to the laboratory in their home cage. An internal cannula (33 gauge) attached via polyethylene tubing to a 10-μl syringe was back-filled with aCSF or drug to eliminate any dead volume and inserted to extend 1 mm below the tip of the guide cannula; blood pressure and heart rate were allowed to return to baseline levels. All injections were unilateral (250 nl in 10 s) and cardiovascular parameters were monitored for at least 30 min. Arterial blood pressure (mm Hg) was calculated for 30-s periods as a mean of 200 data points/s of pulsatile arterial pressure; heart rate (beats/min) was derived for 30-s intervals at a sampling rate of 1 Hz using a tachograph triggered by the pulsatile blood pressure signal. A Grass model 7D polygraph (Grass Instruments, Quincy, MA, USA) and computerized data acquisition (LabTech NOTEBOOK, Laboratory Technology, Wilmington, MA, USA) were used for data collection.

2.3. Drugs

The full 5-HT_{1A} receptor agonist (+)-8-OH-DPAT [*R*-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide] and the non-selective 5-HT receptor antagonist methiothepin (methiothepin mesylate) were purchased from RBI (Research Biochemicals, Natick, MA, USA), dissolved in aCSF (pH 7.6; Mitchell et al., 1963) and prepared fresh daily. Atropine (atropine methyl nitrate), urethane, thionin (all purchased from Sigma, St. Louis, MO, USA) and sotalol (a gift from Brystol-Myers, Evansville, IN, USA) were dissolved in 0.9% NaCl.

2.4. Histology

Rats were anesthetized with urethane (1.2 g/kg i.p.) and 250 nl thionin solution (13 mg/ml) were microinjected in 10 s through the implanted guide cannula for localization of the injection site. The animals were perfused transcardially with 0.9% saline followed by 10% buffered formalin solution. The brains were removed, allowed to fix in the 10% buffered formalin for at least 24 h, cut into 250-μm coronal sections using a Lancer series 1000 vibratome and stained (Riboni et al., 1991). Data only from rats with injection sites within the medial or

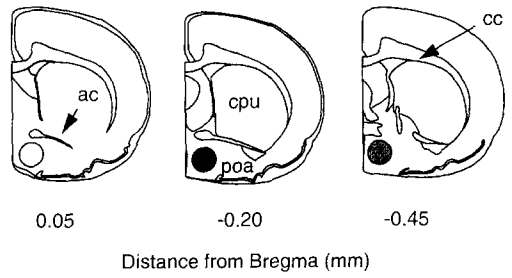

Fig. 1. Schematic representation of microinjection site. Coronal sections of rat brain demonstrating the approximate distribution of dye injection (250 nl/10 s). Data were analyzed only if the darkest staining was localized to the medial and lateral preoptic areas as represented by the black circle in the middle panel. Both the size and shading of the circle are rough estimates of what was observed upon histological examination. The distances from bregma are based on Paxinos and Watson (1986). ac, anterior commissure; cc, corpus callosum; cpu, caudate putamen; poa, preoptic area.

lateral preoptic area (represented schematically in Fig. 1) were analyzed and included in the study.

2.5. Data analysis and statistics

Data are presented as change in blood pressure or heart rate from a pre-injection baseline value or as the area under the curve (AUC) of the responses. Maximal changes in blood pressure and heart rate were calculated as the differences between the pre-injection baseline for each individual rat and the highest 30 s mean value recorded during the 10 min subsequent to drug microinjection. The baseline values of blood pressure and heart rate for all experiments are presented in the tables, figures or figure legends. The AUCs were calculated from the changes in blood pressure and heart rate over the first 10 min following microinjection using the trapezoidal rule to integrate the magnitude and duration of the responses.

Data are expressed as the mean ± S.E.M. determined from 4–13 rats/group. The data were analyzed by one- or two-way analysis of variance (as appropriate) followed post-hoc by Student-Newman-Keuls' test for all pair-wise multiple comparison procedures or Dunnett's test for comparison to the control group. In all cases, differences between groups were considered to be significant at $P < 0.05$.

3. Results

3.1. Histology

Data presented in this study represent responses only from rats where the injection site was limited to the medial and lateral preoptic areas (Fig. 1). The injection of 250 nl of dye normally was observed in two 250-μm sections (the middle and right panels of Fig. 1) with the predominant staining always within 1 coronal section approximately −0.20 mm from bregma. In some rats, there was a trace of dye observed in the preceding section whereas most of the brains contained modest staining in the subsequent section. If dye was in the lateral or third ventricles, centered > 0.75 mm lateral from midline, reaching the anterior commissure or in the section −0.70 mm from bregma, the data were not analyzed.

3.2. Expt. 1: Effects of repeated microinjections into the preoptic area in conscious rats

To determine if repeated microinjections into the preoptic area elicited reproducible responses, aCSF (250 nl) or (+)-8-OH-DPAT (20 nmol/250 nl) was administered 4 times at 48-h intervals. Blood pressure and heart rate were recorded prior to (Table 1) and for 30 min subsequent to each of the treatments. The data, presented as maximal change from the pre-injection baseline, demonstrate that the administration of aCSF into the preoptic area did not produce a significant effect on blood pressure or heart rate following any of the 4 injections (open columns, Fig. 2). By contrast, the 5-HT_{1A} receptor agonist (+)-8-OH-DPAT increased both blood pressure and heart rate as compared to aCSF and baseline on trial 1 (filled columns, Fig. 2). Neither blood pressure nor heart rate responses to (+)-8-OH-DPAT were significantly different from aCSF on trials 2–4. In addition, there were no differences between the responses produced by (+)-8-OH-DPAT on trial 1 when compared to any other trial.

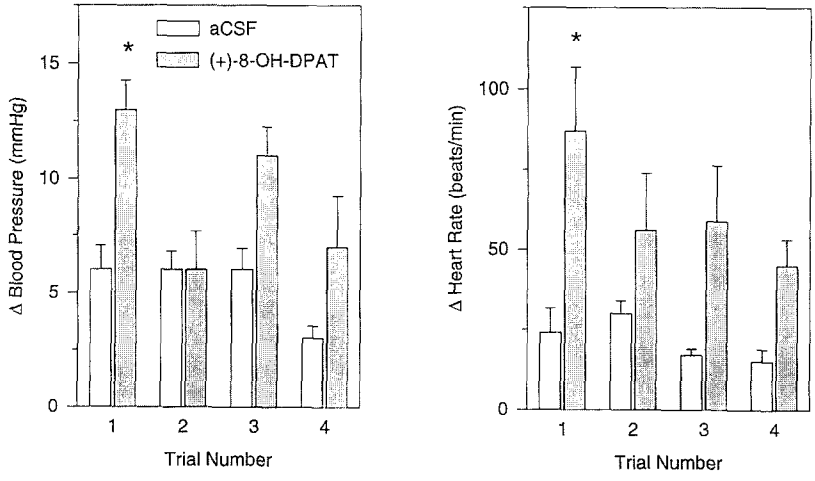

Fig. 2. Maximal changes in blood pressure and heart rate elicited by microinjection of (+)-8-OH-DPAT into the preoptic area of conscious rats diminish upon repeated injection. aCSF (250 nl, $n = 4$) or (+)-8-OH-DPAT (20 nmol, $n = 6$) was injected into the preoptic area of conscious rats 4 times (trials 1–4) with 48 h between trials. Columns represent the mean ± S.E.M. Baseline values of blood pressure and heart rate for each trial are presented in Table 1. * Significantly different from baseline and aCSF for the same trial.

Table 1
Baseline blood pressures and heart rates for data presented in Fig. 2

Trial	Baseline values			
	aCSF		(+)-8-OH-DPAT	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg)	Heart rate (beats/min)
1	99 ± 2	369 ± 20	104 ± 2	377 ± 10
2	108 ± 5	365 ± 19	104 ± 5	384 ± 16
3	102 ± 2	370 ± 17	109 ± 4	403 ± 5
4	100 ± 4	377 ± 20	105 ± 5	371 ± 14

Mean ± S.E.M. values of blood pressure and heart rate determined in conscious rats prior to the microinjection of aCSF or (+)-8-OH-DPAT into the preoptic area on 4 separate trials at 48-h intervals. See legend to Fig. 2 for further details. There were no differences between trial number or treatment ($P > 0.05$).

On trials 2–4, (+)-8-OH-DPAT (20 nmol) tended to produce a mild bradycardia beginning approximately 5 min after microinjection. The maximal decreases occurred around 10 min and were −32 ± 21, −20 ± 20 and −25 ± 11 beats/min following trials 2, 3 and 4, respectively. This bradycardia was rarely observed following the first

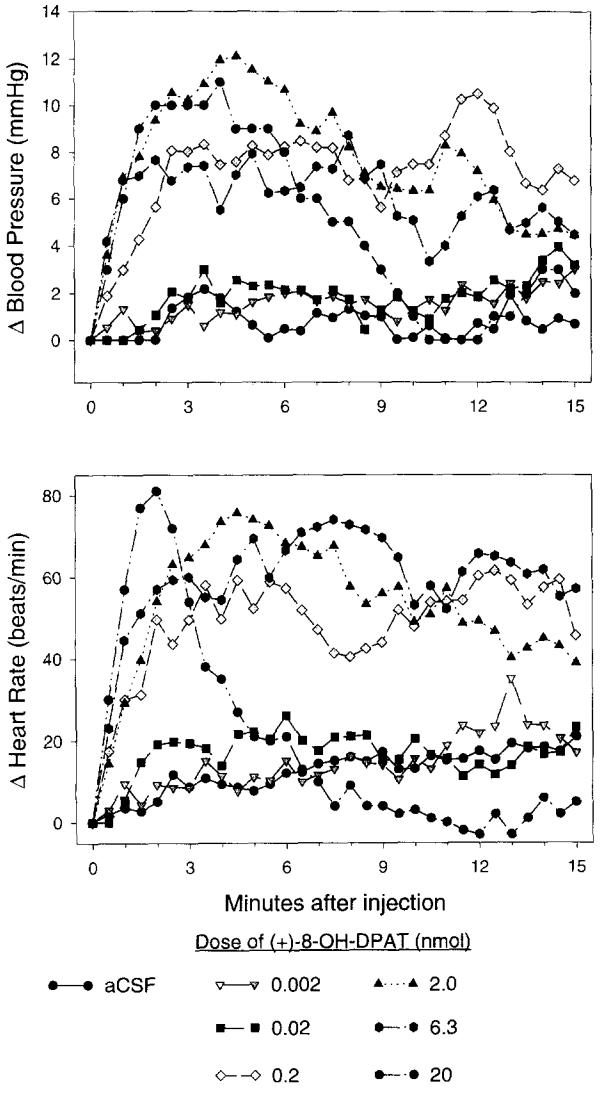

Fig. 3. Descriptive representation of blood pressure and heart rate responses elicited by microinjection of aCSF or a dose (0.002–20 nmol) of (+)-8-OH-DPAT into the preoptic area of conscious rats. Symbols represent the mean change in blood pressure or heart rate at 30-s intervals from $t = 0$ (baseline). See Table 2 for baseline data and quantitative analyses.

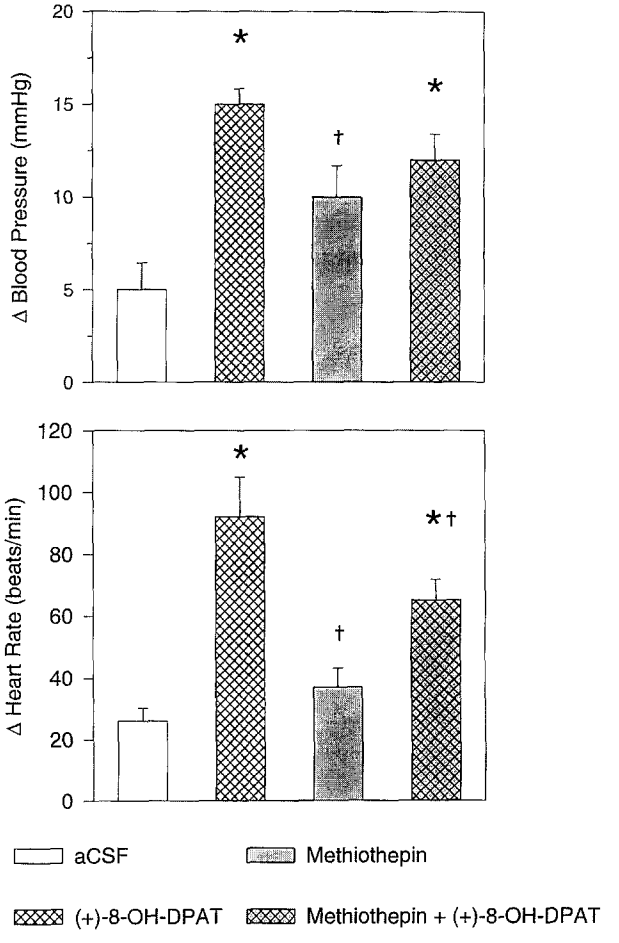

Fig. 4. The non-selective serotonin receptor antagonist methiothepin attenuated the blood pressure and heart rate responses produced by the microinjection (+)-8-OH-DPAT into the preoptic area of conscious rats. Rats were injected with aCSF (250 nl; $n = 8$), (+)-8-OH-DPAT (0.2 nmol, $n = 12$), methiothepin (1 nmol, $n = 11$) or the combination of methiothepin (1 nmol) + (+)-8-OH-DPAT (0.2 nmol, $n = 11$). The data represent the maximal changes from baseline. The baseline values (blood pressure in mm Hg and heart rate in beats/min) for the 4 treatment groups were: (1) aCSF; 106 ± 2 and 380 ± 7; (2) (+)-8-OH-DPAT; 111 ± 1 and 382 ± 7; (3) methiothepin; 115 ± 3 and 384 ± 5; and (4) the combination; 110 ± 2 and 401 ± 5. * Significantly different from aCSF. † Significantly different from (+)-8-OH-DPAT.

Table 2
Blood pressure and heart rate responses elicited by microinjection of aCSF and graded doses of (+)-8-OH-DPAT (in nmol) into the preoptic area of conscious rats

	Baseline		Maximal change		AUC	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg × min)	Heart rate (beats/min × min)
aCSF	118 ± 3	414 ± 10	5 ± 1	26 ± 4	5 ± 10	91 ± 27
(+)-8-OH-DPAT						
0.002	127 ± 6	423 ± 16	5 ± 1	31 ± 6	13 ± 8	106 ± 45
0.02	129 ± 4	422 ± 12	7 ± 2	37 ± 8	15 ± 8	171 ± 72
0.2	127 ± 7	408 ± 19	15 ± 1 [a]	92 ± 13 [a]	67 ± 6 [a]	450 ± 71 [a]
2	115 ± 4	423 ± 12	17 ± 2 [a]	92 ± 16 [a]	90 ± 17 [a]	576 ± 130 [a]
6.3	116 ± 2	388 ± 13	16 ± 2 [a]	103 ± 14 [a]	67 ± 23 [a]	604 ± 140 [a]
20	114 ± 1	430 ± 12	13 ± [a]	87 ± 8 [a]	68 ± 5 [a]	290 ± 45

Conscious rats were injected with aCSF (250 nl) or 1 dose of (+)-8-OH-DPAT into the preoptic area. The time-courses are presented in Fig. 3. Data were calculated as described in Section 2 and are expressed as mean ± S.E.M. as determined from 6–12 rats/group.
[a] Significantly different from aCSF.

injection of (+)-8-OH-DPAT. Since it appeared that the responses to (+)-8-OH-DPAT decreased and/or changed upon repeated injections, rats were used only 1 time in all subsequent experiments.

3.3. Expt. 2: Time-course following the microinjection of graded doses of (+)-8-OH-DPAT into the preoptic area in conscious rats

The time-course for the effects of graded doses of (+)-8-OH-DPAT administered into the preoptic area of conscious rats on blood pressure and heart rate is presented descriptively in Fig. 3. The pressor and tachycardic responses were maximal following the microinjection of 2 nmol (Table 2), peaked 4–7 min after drug microinjection and were maintained above control for 10–15 min. The peak responses reported in Table 2 are greater than the values at any time-point presented in Fig. 3. This was due to variation in the time at which the peak responses occurred in individual rats, which is the value used to calculate the mean presented in the table (see Section 2.5 for description of calculations). The administration of 20 nmol (+)-8-OH-DPAT produced an increase in blood pressure that was not statistically distinguished from the 2 lower doses as determined by maximal changes or AUC (Table 2). Similarly, 2, 6.3 and 20 nmol produced equivalent maximal increases in heart rate. However, when expressed as AUC the tachycardia elicited by the highest dose was less than that produced by either 2 or 6.3 nmol (Table 2) due to a shorter duration of the response (see Fig. 3).

3.4. Expt. 3: effects of (+)-8-OH-DPAT microinjected into the preoptic area in anesthetized rats

Administration of (+)-8-OH-DPAT into the preoptic area increased motor activity as assessed subjectively. To determine if the cardiovascular responses might be secondary to behavioral activation, (+)-8-OH-DPAT (2 nmol) was administered into the preoptic area of urethane-anesthetized rats. The 5-HT_{1A} receptor agonist increased heart rate but not blood pressure when compared to aCSF-injected controls (Table 3). The maximal increase in heart rate occurred within 3–9 min after microinjection and was sustained for approximately 10 min. The rats remained deeply anesthetized throughout the duration of the experi-

Table 3
Blood pressure and heart rate responses elicited by microinjection of aCSF and 2 nmol (+)-8-OH-DPAT into the preoptic area of urethane-anesthetized rats

	Baseline		Maximal change		AUC	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg × min)	Heart rate (beats/min × min)
aCSF	114 ± 8	483 ± 9	1 ± 1	14 ± 1	−2 ± 6	11 ± 18
(+)-8-OH-DPAT	118 ± 5	477 ± 14	8 ± 2	31 ± 8 [a]	40 ± 14	199 ± 65 [a]

Urethane-anesthetized rats were injected with aCSF (250 nl, $n = 4$) or (+)-8-OH-DPAT (2 nmol, $n = 5$) into the preoptic area. Each rat was used only for 1 drug injection. Data were calculated as described in Section 2 and are expressed as mean ± S.E.M.
[a] Significantly different from aCSF.

ment. Thus, although some of the response produced by (+)-8-OH-DPAT might be secondary to the behavior, microinjection of the 5-HT_{1A} receptor agonist clearly elicited tachycardia in the absence of movement.

3.5. Expt. 4: effects of the 5-HT receptor antagonist methiothepin in conscious rats

In a pilot study, the microinjection of the non-selective 5-HT receptor antagonist methiothepin in doses up to 1 nmol into the preoptic area did not produce significant cardiovascular responses. At ≥ 10 nmol, increases in blood pressure and heart rate were observed (data not presented).

To determine if methiothepin would decrease the response elicited by (+)-8-OH-DPAT, aCSF, (+)-8-OH-DPAT (0.2 nmol), methiothepin (1 nmol) or methiothepin plus (+)-8-OH-DPAT (1 and 0.2 nmol, respectively) were administered as a single 250 nl microinjection into the preoptic area (Fig. 4). The 5-HT_{1A} receptor agonist (+)-8-OH-DPAT increased both blood pressure and heart rate whereas methiothepin by itself did not alter either parameter. The blood pressure response elicited by the simultaneous microinjection of the 2 drugs was greater than aCSF, but not different from either drug alone (top panel, Fig. 4). The microinjection of methiothepin plus (+)-8-OH-DPAT increased heart rate when compared to either aCSF or methiothepin, however, the tachycardia elicited by the combination was significantly less than that produced by (+)-8-OH-DPAT (bottom panel, Fig. 4). Thus, methiothepin attenuated but did not eliminate cardiovascular responses elicited by (+)-8-OH-DPAT.

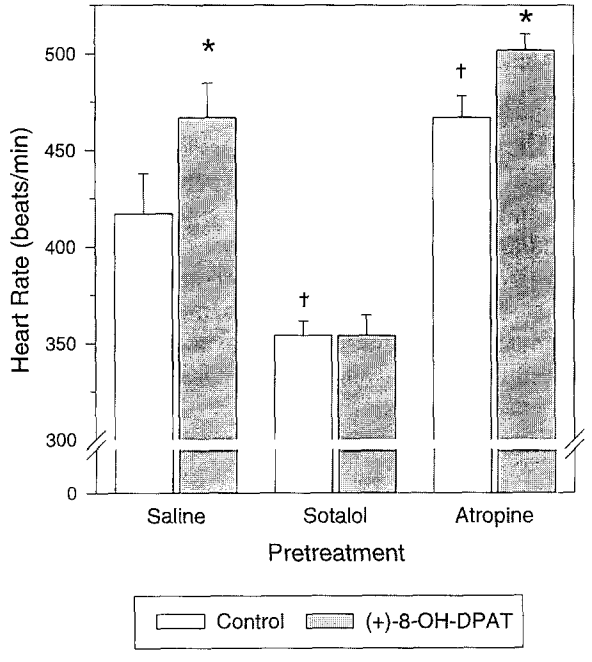

Fig. 5. Sotalol, but not atropine, blocked the tachycardia produced by the microinjection of (+)-8-OH-DPAT into the preoptic area of conscious rats. Rats were injected intravenously with saline (100 μl/kg, $n = 7$), sotalol (10 mg/kg, $n = 8$) or atropine (1 mg/kg, $n = 7$). 5 min later, heart rate was determined (Control, open columns) and then all rats received (+)-8-OH-DPAT (2 nmol) into the preoptic area. Maximal values within the subsequent 10 min were recorded (filled columns). *Significantly different from appropriate Control value. †Significantly different from Saline/Control value.

3.6. Expt. 5: effects of autonomic receptor antagonists on (+)-8-OH-DPAT-induced tachycardia in conscious rats

To determine if the tachycardia elicited by (+)-8-OH-DPAT in the preoptic area was produced by sympathoadrenal stimulation or by vagal withdrawal, rats were pretreated i.v. with either the peripheral β-adrenoceptor receptor antagonist sotalol (10 mg/kg) or the peripheral muscarinic receptor antagonist atropine (1 mg/kg). Predictably, sotalol decreased and atropine increased heart rate (open columns, Fig. 5). When administered 5 min after the autonomic receptor antagonists, (+)-8-OH-DPAT (0.2 nmol) increased heart rate in saline- and atropine-pretreated rats, but not in those rats pre-treated with sotalol (Fig. 5). This suggests that (+)-8-OH-DPAT acts within the forebrain to increase heart rate by sympathoadrenal activation.

4. Discussion

The experiments presented in this study demonstrate that activation of 5-HT_{1A} receptors by the microinjection of (+)-8-OH-DPAT into the preoptic area causes slight, variable increases in blood pressure and robust, consistent increases in heart rate in conscious, freely moving rats. The tachycardia is mediated by sympathoadrenal activation unrelated to changes in overt behavior and the hypertension similarly may be due to sympathetic activation or may be, in part, secondary to behavioral arousal as it was absent in urethane-anesthetized rats. Our studies did not assess the role of the sympathetic nervous system in the pressor response to (+)-8-OH-DPAT.

The experiments were initiated in response to the hypothesis put forth that activation of 5-HT_{1A} receptors in the forebrain can produce hypertension and tachycardia. This hypothesis was derived indirectly from 2 observations: (1) the administration of serotonin and selective 5-HT_{1A} receptor agonists into the lateral cerebral ventricle at appropriate doses increases blood pressure, heart rate (Anderson et al., 1992; Dedeoğlu and Fisher, 1991, 1994) and renal nerve activity (Anderson et al., 1992) in rats; and (2) the well-established role for midbrain and brainstem 5-HT_{1A} receptors to inhibit sympathetic and excite vagal outflow from the central nervous system (McCall and Clement, 1994). Within the rat forebrain, 5-HT_{1A} receptors have been found in low to moderate densities in various hypothalamic nuclei, the preoptic area and the bed nucleus of the stria terminalis (Chalmers and Watson, 1991; Pom-

peiano et al., 1992). With that in mind and with the knowledge that serotonin can elicit cardiovascular responses in the preoptic area (Kuhn et al., 1980; Smits et al., 1978; Smits and Struyker-Boudier, 1976; Wolf et al., 1981), it seemed a likely site to examine. It was found was that (+)-8-OH-DPAT, a highly selective 5-HT_{1A} receptor agonist, when microinjected into the preoptic area in a volume of 250 nl produced cardiovascular responses that were attenuated by a non-selective 5-HT receptor antagonist, methiothepin. These observations are similar to those reported previously following the administration of relatively low doses of (±)-8-OH-DPAT into the lateral cerebral ventricle of conscious rats (Dedeoğlu and Fisher, 1991). Thus, the current data confirm the hypothesis put forth by others that activation of forebrain 5-HT_{1A} receptors can produce hypertension and tachycardia.

Although the distribution of the drug was not determined, it is felt to be limited to a sphere of tissue substantially < 1 mm in diameter based on several factors. First, the injection of an equivalent volume of dye over the same 10 s routinely appeared in only 2–3 coronal sections of approximately 250 μm each. Thus, the diffusion of the dye was limited to a sphere of not more than 300–400 μm in diameter. Second, 20 nmol (+)-8-OH-DPAT in 250 nl aCSF did not alter blood pressure or heart rate when microinjected into the anterior hypothalamic area (B.L. Butz and R.H. Alper, unpublished observations). Apparently, < 1% of the drug diffused the 1.5 mm distance between the anterior hypothalamus and the preoptic area since as little as 0.2 nmol increase blood pressure and heart rate when applied to the preoptic area. Third, if (+)-8-OH-DPAT had diffused into the third ventricle (only 750 μm from the center of the injection site), the responses would have been a slight hypotension and a marked bradycardia (Dedeoğlu and Fisher, 1991; Alper and Schmitz, 1996). Although such responses were not observed, diffusion of (+)-8-OH-DPAT into the CSF or to the brainstem by some other route might have contributed to the biphasic nature on heart rate elicited by the highest dose. Finally, in a study designed to determine the effect of volume and rate of injection on the diffusion of [^{3}H]bicuculline when microinjected into the paraventricular nucleus, Segura et al. (1992) found that neither variable was particularly critical to the extent of the distribution of the drug. Although it cannot be stated with certainty that the preoptic area is the precise site of action in the current study, it is without a doubt that activation of 5-HT_{1A} receptors in the forebrain, most likely in the preoptic area or a region within approximately 500–750 μm of the preoptic area, such as the bed nucleus of the stria terminalis, increases blood pressure and heart rate.

The cardiovascular response did not demonstrate a clear dose-response relationship. That is, in doses ranging from 0.2 to 20 nmol a slight increase in blood pressure and a more substantial increase in heart rate were observed although there were no statistical differences among the doses. The microinjection of 2 lower doses (+)-8-OH-DPAT or vehicle (250 nl of aCSF) into the preoptic area did not affect the cardiovascular system, thus, the responses were not injection artifacts. Since the tachycardia was attenuated by the concomitant microinjection of the non-selective 5-HT receptor antagonist methiothepin, we can further conclude that the increase in heart rate was receptor-mediated and not simply a response to the application of a hypertonic solution. If the response had been to the solute unrelated to 5-HT_{1A} receptor activation, the combination of (+)-8-OH-DPAT (0.2 nmol) and methiothepin (1 nmol) would have elicited a tachycardia similar to or greater than (+)-8-OH-DPAT (0.2 nmol) alone. Although it is probable that 5-HT_{1A} receptors are involved due primarily to the high degree of specificity of (+)-8-OH-DPAT for that receptor, a role for the 5-HT_7 receptor cannot be ruled out (Bard et al., 1993; Tsou et al., 1994).

The mechanism by which microinjection of (+)-8-OH-DPAT into the preoptic area increases heart rate involves activation of cardiac and/or adrenal sympathetic nerves. This is based on the observation that the peripheral β-adrenoceptor receptor antagonist sotalol (Antonaccio and Gomoll, 1988) completely abolished the tachycardia. It appears unlikely that (+)-8-OH-DPAT produced a marked generalized sympathetic stimulation since the increase in blood pressure was relatively small, very variable and may have been secondary to behavioral activation (it was abolished in urethane-anesthetized rats). It is as yet unknown if the sympathetic nervous system participates in the pressor response.

In closing, it was found that the microinjection of the selective 5-HT_{1A} receptor agonist (+)-8-OH-DPAT directly into the preoptic area of conscious rats produced time-dependent increases in blood pressure and heart rate. The tachycardia was clearly was mediated by sympathoadrenal activation whereas the increase in blood pressure might have been due to increased sympathetic outflow or secondary to behavioral arousal. These data provide the first direct evidence that 5-HT_{1A} receptors in the preoptic area and/or adjacent forebrain structures participate in central cardiovascular regulation. It is important to note that these 5-HT_{1A} receptors produce cardiovascular and underlying autonomic responses that are opposite to those commonly associated with midbrain and brainstem 5-HT_{1A} receptor activation. The functional importance of forebrain 5-HT_{1A} receptors in cardiovascular physiology and pharmacology is yet to be determined.

Acknowledgements

We would like to express their appreciation for the excellent technical assistance from Mr. Todd M. Schmitz and Ms. Amanda Bell during the course of these experiments. The research was supported in part by a Grant-in-Aid from the Kansas Affiliate of the American Heart

Association (KS-92-GS-46) and also by NS 29765 from the National Institutes of Health.

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